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SOIL AMENDMENTS WITH ORGANIC MATTER FOR THE CONTROL OF HOLLOW STALK (*Pectobacterium carotovorum subsp. carotovorum*) OF BESUKI CIGAR TOBACCO

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ABSTRACT

Amendments of soil with organic matter have been known to provide control of soilborne pathogens and to improve soil properties as well. Four sources organic matter, viz: rice straw, neem cake, chicken, and cow manure were amended one month prior to planting in soil naturally infested by *Pectobacterium carotovorum* subsp. *carotovorum*, the causal agent of hollow stalk of tobacco. Soil without added organic matter served as control and seedlings treated with streptomycin sulphate was used as a comparison. This treatment was combined with one of two sources of fertilizer N viz:, urea, and $CaNO_3$. The field experiment was conducted in North Jember arranged in randomized blocked factorial with three replicates. The chicken manure amendment gave the best control of hollow stalk with lowest disease severity (12.03%) compared to other organic matter treatments or even control (31.31%). Chicken manure also improved plant height, yield, and the quality of flue cured tobacco. All organic matter treatments increased soil microbial populations of fungi, bacteria, and actinomycetes. This may be related to the suppression of the pathogen and the consequent reduction of disease severity. Treat-ment of soil with urea or $CaNO_3$ showed no effect on disease severity or growth or quality of tobacco.

Keywords: organic matter amendments, hollow stalk, *Pectobacterium carotovorum subsp. carotovorum*

INTRODUCTION

Hollow stalk of tobacco caused by *Pectobacterium carotovorum* subsp. *Carotovorum* (previously known as *Erwinia carotovora* subsp *carotovora*) is a major disease of cigar tobacco in Jember (East Java of Indonesia). The first symptom appears on the upper part of the plant, particularly after topping and sucker-ing, during the rainy season. The upper leaves start to wilt followed by stalk rot and the pith disintegrating to form a hollow cavity. The bacteria enter the plant through topping wounds then moving to disintegrate the pith leading to the development of the hollow stem. Dalmadiyo (1999) reported that in some Besuki Cigar Tobacco (BCT) plants the symptom appeared from the lower part of the stem. These symptoms are similar to those of greenville wilt caused by *Ralstonia solanacearum* with the plants wilting on one side followed by a brown rot and hollowing of the lower stem.. *Erwinia* also affects tobacco leaf during the curing process causing soft rot and the consequent drop in the quality of tobacco.

The use of the bactericide streptomycin sulphate, commonly recommended for the management of this disease is expensive and in addition is often unavailable to growers in the local market. This situation led the tobacco growers to use excessive use of inappropriate pesticides. As a result, the disease has spread widely in almost all of the cigar tobacco growing areas in Jember with various degrees of severity. To overcome the problem, an alter-native disease management strategy based on an ecological approach, such as the use of organic matter amendment is urgently needed.

Murdiyati (personal communication) observed that soil in Jember contains calcium at levels low to medium needed to support tobacco growth. This condition has worsened with the current use of KNO_3 fertilizer to substitute $CaNO_3$. As a result, there has been a drop in the quality of tobacco because of the develop-ment of the '*glassy*' symptom on tobacco leaves. The glassy

symptom appears to be related to the failure of the leaf to form sturdy middle lamella, critical to the architecture of the tobacco leaf , due to calcium deficiency. Bain *et al.* (1996) examined the role of gypsum on the development of soft rot of potato caused by *Erwinia carotovora*. They noticed that the spread and disease severity was high in plots without $CaSO_4$ treatment. Furthermore, Schober and Vermeulen (1999) reported that the disease severity of chicory soft rot increased when N fertilizer was added, but decreased with the addition of Ca. Pectinolytic activities of *P. carotovorum* are known to be affected by treatments with Ca and N. The addition of calcium methasolate 0.5% in the water reduced the infection process of *E. carotovora* on asparagus. Flego *et al.* (1997) stated that increase in the concentration of calcium in bacterial micro-environment inhibited the enzyme pehA endopolygalacturonase, an important enzyme produced during the early stage of infection by the bacteria resulting in the loss of its virulence. The addition of calcium to plant is considered to increase the structure and integrity of plant cell wall and rendering the plant was more resistant to pathogen infection (McGuire and Kelman, 1984). The addition of calcium can also enhance the production of extracellular enzymes which can also function as an infection signal (Messiaen *et al.,* 1993)

This paper reports the results of a study on the potential of several sources of organic matter and two sources of N to sppress the development of the on hollowstalk disease and the growth and yield of tobacco .

MATERIALS AND METHODS

This study was conducted in 2008, in North Jember (East Java-Indonesia), where Besuki Cigar Tobacco is grown on a large scale and the soil is naturally infested with *P. carotovorum subsp. carotovorum.* The sources of organic matter tested were 10 ton ha^{-1} of rice straw, cow manure, chicken manure, and neem (*Azadiracta indica*)cake. The organic matter was incorporated into the soil one month before planting. Streptomycin sulphate (used at the rate of 2g l^{-1}) was used as a comparison. The bactericide was applied before planting by soaking the seedlings over-night. The source of N used were urea or Ca NO3 at 400 kg $ha^{-1.}$. The treatments were arranged in a randomized factorial design with three replicates.

Disease severity was assesed weekly, starting at 14 d after transplanting using the formula : I= D/N x 100%. I being the degree of disease severity, D the number of infected plants, and N the number of all plants assessed in a plot. Populations of soil microorganisms was estimated using selective media (give details of media)1, 2 and 3 months after organic matter incorporation. The soil was sampled at 20-25 cm depth from five random spots in each plot. The growth of tobacco plant was measured every month, represented by 10 plants for each plot. Yield and quality assessments were done after harvest of tobacco leaves. Classification of tobacco filler quality after curing process can be separatedinto five groups based on the length and colour of flue cured leaves. The commercial value of each group of flue cured leaves can be different. Quality A was the most expensive, whilst quality D, the cheapest. Quality R (Residue) was marked by small size flue cured leaves with spots the colour not uniform or the texture too dry due to inappropriate processing. The quality R is normally not acceptable for filler. In this study quality A to D was not significantly different, hence the quality of flue cured material was divided into two group, i.e GOOD (A-D) and RESIDUE (R).

The data were analysed using ANOVA, followed by LSD test at 5%.

RESULTS AND DISCUSSION

Disease Severity

Addition of organic matter significantly reduced disease severity upto 10 weeks after transplanting (wap). Chicken manure was the best among the organic matter screened for the control of hollow stalk, while, streptomycin sulphate gave better control compared to rice straw (Table 1). Addition of calcium and N in the form of $CaNO_3$ did not significantly affect the severity of disease. The interaction effect on disease suppression occurred only up to 5 weeks after transplanting, and no interaction effects were evident during 6-10 weeks (Table 2). Plant growth responses to the treatments are presented in figure 1.

Rate of development of hollow stalk disease was slower in plots treated with organic matter, with the exception of rice straw with $CaNO_3$ (Figures 2 and 3).

Growth, Yield, and Quality of Tobacco

All of the tested organic matter treat-ments, with the exception of rice straw, increaseed plant height. The growth of the plant was highest when the soil was treated with chicken manure (115.2 cm), followed by neem cake (103.18 cm), and cow manure (96.05 cm) (Table 3). The growth of the plant was the least (78.6 cm) in this treatment as the plants were heavily infected by pathogen and had wilted.

Addition of calcium or urea did not significantly affect the height of the plant, although urea tended to increase the growth (93.94 cm) compared to $CaNO_3$ (90.16cm)

Table 1 Effect of the addition of organic matter on hollow stalk disease severity on tobacco at 10 weeks after planting

Organic matter source	Disease severity*)
Rice straw	19.57 b
Cow manure	15.30 ab
Neem cake	14.81 ab
Chicken manure	12.03 a
Sterptomycin sulphate	15.97 ab
Control (No organic matter)	31.31 c
LSD (5%)	5.85

Remarks: *) The data were Arcsin Vx+0.5 transformed. Values followed by the same letter were not significantly different at 5% LSD test

Table 2 Effect of the addition of organic matter and $CaNO_3$ or urea on hollow stalk disease severity on tobacco 2-5 weeks after planting (WAP)

Organic matter source	2 wap		3 wap		4 wap		5 wap	
	$CaNO_3$	Urea	$CaNO_3$	Urea	$CaNO_3$	Urea	$CaNO_3$	Urea
Rice straw	11.71	13.45	12.69	14.75	12.69	16.64	12.69	16.64
Cow manure	12.69	7.29	13.13	7.29	13.97	7.29	13.97	7.29
Neem cake	12.04	8.29	12.42	9.46	12.77	10.03	12.77	10.03
Chicken manure	6.73	9.66	7.42	11.64	7.42	11.64	7.98	11.64
Streptomycin sulphate	7.73	9.66	8.41	11.93	10.31	12.62	10.31	12.62
Control	16.16	17.19	17.92	18.14	18.54	19.30	18.88	19.30
LSD (5%)	3.57		4.06		5.19		5.19	

Remarks: *) The data were Arcsin Vx+0.5 transformed. Values followed by the same letter were not significantly different at 5% LSD test

Table 3. Effect of the amendment of soil with organic matter on tobacco plant height

Organic matter source	Plant height (cm)
Rice straw	82.58 a*)
Cow manure	96.05 b
Neem cake	103.18 bc
Chicken manure	115.20 c
Streptomycin sulphate	81.80 a
Control (No organic matter)	73.48 a
LSD (5%)	12.76

Remarks: *) Values followed by the same letter were not significantly different at 5% LSD test.

Control

Streptomycin Sulphate

Cow Manure

Chicken Manure

Rice Straw

Neem Cake

Figure 1. Performance of tobacco plants grown on soil amended with different sources of organic matter

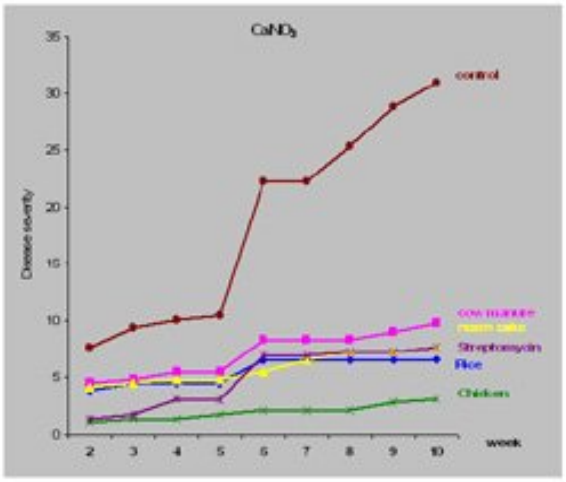

Figure 2. Rate of development of hollow stalk disease of tobacco on plots amended with different sources of organic matter and $CaNO_3$

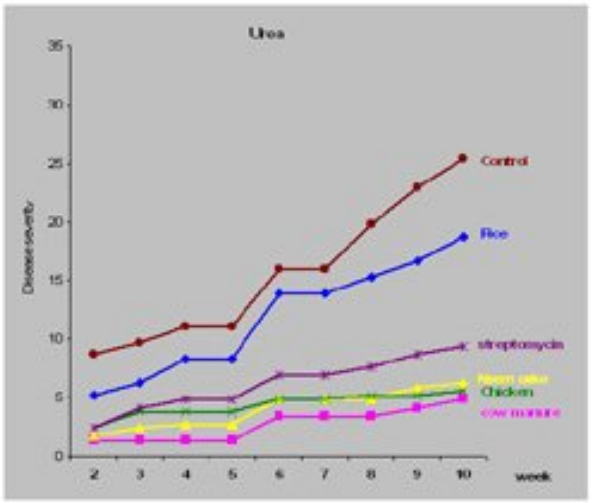

Figure 3. Rate of development of tobacco hollow stalk on plots amendedwith different sources of organic matter and Urea

Addition of organic matter increased yield of fresh tobacco leaf. The highest yield was reached when the plants were treated with chicken manure (13.39 ton ha^{-1}), followed by cow manure (11.38 ton ha^{-1}) and neem cake (11.16 ton ha^{-1}). Yield of leaves in plots treated with rice straw was not significantly different with those in plots treated with streptomycin sulphate. The lowest yield was on plots without organic matter or streptomycin sulphate treatment (7.55 ton ha^{-1}), since the growth was severely affected by pathogen attack or death.

In this study, neither calcium nor urea affected the fresh tobacco leaf production nor the tobacco quality.There were no interaction between the organic matter source and calcium treatment.

Population Dynamics of Soil Microbial Populations

Addition of organic matter enhanced populations of fungi, bacteria, and actinomycetes significantly. A month after incorporation of the organic matter, the highest population of fungi occurred in soil amended with neem cake, followed by rice straw, chicken manure, and cow

manure. The populations of fungi in all treatments decreased within two months after incorporation of amendments, since the soil was dry due to the lack of rain. The highest population of fungi was in soil added with neem cake and rice straw, with the population very low in soil amended with chicken or cow manure, three months after amendment (Figure 4).

The highest actinomycetes and bacterial populations were found in soil amended with chicken and cow manure, one month after incorporation. The population of bacteria in both treatments decreased significantly within 2-3 months after incorporation (Figure 5). The population of actinomycetes decreased in soil amended with chicken or cow manure, within three months, but remained high in soil amended with neem cake or rice straw (Figure 6).

Addition of organic matter, particularly chicken manure, provided significant control of *P. carotovorum subsp. carotovorum* and also increased yield and quality of besuki cigar tobacco. As Stone *et al.* (2004) claimed, organic wastes, including manures, could improve soil quality and plant health. Organic matter also provides long-term suppression of plant diseases caused by soilborne plant pathogens by stimulating the growth of resident soil microflora (Hoitink and Ramos, 2009) which may act as antagonists of the pathogen (Linderman, 1989), reducing the inoculum potential of the pathogen. Roget (2006) reported that the higher microbial population the greater the degree of disease suppression. Gorissen *et al.* (2004) reported that application of swine manure increased soil suppressiveness to the pathogen *Ralstonia solanacearum* through shifting the bacterial community in the soil. They found that population of the pathogen within the amended soil declined slowly from millions colony forming unit to only ten-thousands of colony forming units per gram of soil within 9 week, leading to a decrease in the severity of potato rot. They added that the effect could be enhanced by combining soil solarization to the swine manure treatment.

Almost all sources of organic matter tested, with the exception ofrice straw, increased plant growth and fresh tobacco leaf yield. Gardner and Morgan (1993) proposed that, organic matter increased plant growth, root vigor and cholorophyll synthesis leading to increased production tobacco. This is because organic matter amendments improves not only the structure, physical and chemical properties of the soil but also provides nutrients for plant growth through decomposition processes (Reganold, 1988).

Organic matter amendment therefore not only increases the activity in the soil of antagonists of the pathogen, but also increases plant resistance to the pathogen through root vigor and enhaced resistance of the host to the disease.

Although there are many reports indicating the ability of calcium to inhibit the growth and reduce the virulence of *Pectobacterium* or *R.solanacearum*, our study did not show this effect of calcium on disease suppression. This difference may be related to the concentration of calcium used or to other biotic or abiotic soil factors involved. The role of Ca in disease amlioration may be complex.

Table 4. Effect of the addition of organic matter on Fresh Tobacco Yield, Quality of Flue cured (Good and Residue) leaves

Organic matter source	Fresh Yield ton ha^{-1})		GOOD flue cured (kg ha^{-1})		RESIDU flue cured (kg ha^{-1})
Rice straw	9.67	B	965.63	b	179.17
Cow manure	11.38	C	973.33	b	225.00
Neem cake	11.16	C	1085.83	bc	183.33
Chicken manure	13.39	D	1262.92	c	243.75
Streptomycin sulphate	9.47	B	702.92	a	233.33
Control (No organic matter)	7.55	A	573.13	a	162.50
LSD (5%)	1.34		258.80		tn

Remarks= *) Values followed by the same letter were not significantly different at 5% LSD test

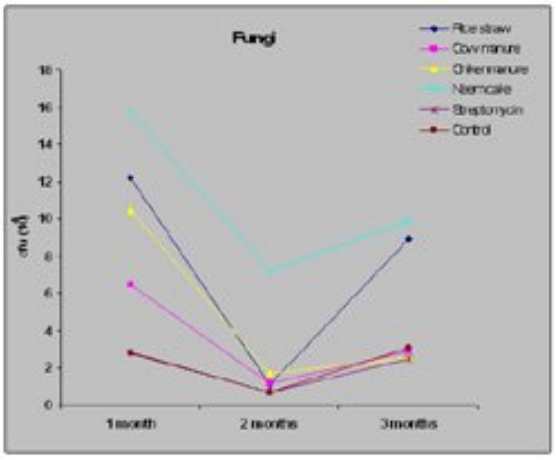

Figure 4 Population dynamics of fungi in soil amended with different sources of organic matter

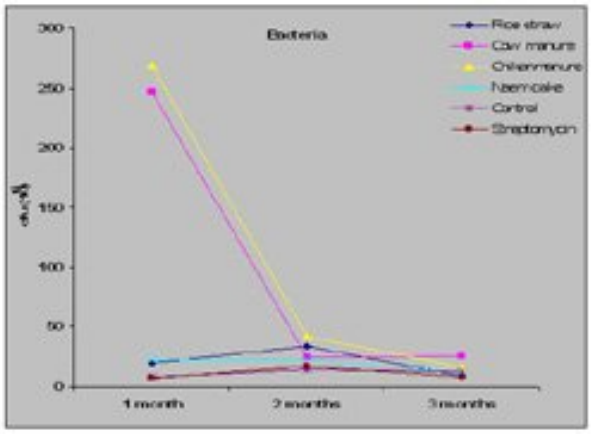

Figure 5 Population dynamics of bacteria in soil ameded with different sources of organic matter

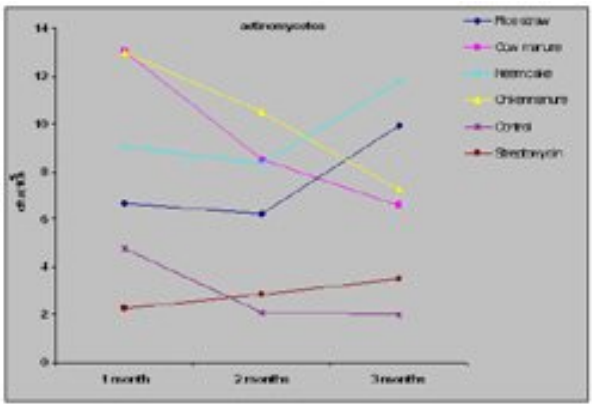

Figure 6 Population dynamics of actinomycetes in soil amended with different sources of organic matter

Vudivanich (2002) found that the addition of urea and calcium oxide to *R. solanacearum* infested soil, three weeks before planting ginger, reduced population of the pathogen in the soil. Decomposition of urea to ammonium, ammonia and nitrate in high pH soil environment was toxic to *R.solanacearum*. Yamazaki *et al.* (2000 a, b) reported that there was a correlation between tomato plant resistance to *R. solanacearum* and calcium adsorption and concentration in plant tissue. However, its role in disease suppression was not investigated.

CONCLUSIONS AND SUGGESTION

Organic matter amendments, particularly chicken manure, is potentially effective in controlling hollow stalk disease tobacco, caused by *P. carotovorum subsp. carotovorum* and in addition was found to stimulate plant growth resulting in increased yield and quality of Besuki Cigar Tobacco under the field conditions tested.

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